

# Synthesis of Capsaicin Oligosaccharides and Their Anti-Allergic Activity

—Synthesis of Capsaicin Oligosaccharides as Anti-Allergic Food-Additives

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Received August 9, 2011; revised September 16, 2011; accepted September 27, 2011

## ABSTRACT

The production of  $\beta$ -maltooligosaccharides of capsaicin was investigated using *Lactobacillus delbrueckii* and cyclodextrin glucanotransferase (CGTase) as biocatalysts. The cells of *L. delbrueckii* glucosylated capsaicin to give its  $\beta$ -glucoside. The  $\beta$ -glucoside of capsaicin was converted into the corresponding  $\beta$ -maltoside and  $\beta$ -maltotrioside by CGTase. On the other hand,  $\beta$ -melibioside and  $\beta$ -isomaltoside of capsaicin, which were two new compounds, were synthesized by chemical glycosylation. The  $\beta$ -glucoside,  $\beta$ -maltoside,  $\beta$ -melibioside, and  $\beta$ -isomaltoside of capsaicin showed inhibitory effects on IgE antibody production.

**Keywords:** Glycosylation; Capsaicin; *Lactobacillus delbrueckii*; CGTase; Anti-Allergic Activity

## 1. Introduction

Plants of the *Capsicum* species have been important sources of food, spices, and medicines for centuries worldwide. Capsaicin, *N*-[(4-hydroxy-3-methoxyphenyl)methyl]-8-methyl-(*E*)-6-nonenamide, is the most pungent principle among naturally occurring capsaicinoids. It has also been reported to decrease adipose tissue weight and serum triacylglycerol content in rats by enhancing energy metabolism [1]. Capsaicin has shown a wide range of pharmacological properties, such as analgesic, antigenotoxic, antimutagenic, and anticarcinogenic effects, and has been used to treat various peripheral painful conditions, including rheumatoid arthritis and diabetic neuropathy [2-5]. However, capsaicinoids possess extensive neurological toxicity, and direct irritant effects on skin and mucous membrane [6]. Furthermore, capsaicinoids are scarcely soluble in water and poorly absorbed after oral administration. These disadvantages prevent capsaicinoids from being used as food additives and medicines.

Glycosylation allows the conversion of water-insoluble and unstable organic compounds into the corresponding water-soluble and stable ones to improve their bioavailability and pharmacological properties [7-16]. In addition, glycosides of functional food-ingredients have been, recently, reported to show anti-allergic activity [17-19]. This paper describes the production of capsaicin maltooligosaccharides, and capsaicin  $\beta$ -melibioside and  $\beta$ -iso-

maltoside, which were two new compounds, by biocatalytic or chemical glycosylation. We also report the anti-allergic activity, *i.e.*, inhibitory effects on IgE antibody formation, of the capsaicin oligosaccharides.

## 2. Experimental

### 2.1. General

Capsaicin was purchased from Aldrich Chemical Co. The <sup>1</sup>H and <sup>13</sup>C nuclear magnetic resonance (NMR), H-H correlation spectroscopy (COSY), C-H COSY, and heteronuclear multiple-bond correlation (HMBC) spectra were recorded in CD<sub>3</sub>OD using a Varian XL-400 spectrometer (Varian Inc.). The chemical shifts were expressed in  $\delta$  (ppm) referring to tetramethylsilane. The fast atom bombardment mass spectrometry (FABMS) spectra were measured using a JEOL MStation JMS-700 spectrometer (JEOL Ltd.). High performance liquid chromatography (HPLC) was carried out on a YMC-Pack R & D ODS column (150 mm  $\times$  30 mm) at 25°C [solvent: methanol-water (9:11, v/v); detection: UV (280 nm); flow rate: 1.0 ml/min].

### 2.2. Synthesis of Capsaicin $\beta$ -Maltoside and $\beta$ -Maltotrioside

Culture medium used for growth of *L. delbrueckii* subsp. *bulgaricus* (Okayama University of Science) had the following composition (in grams per liter): 20 g of lactose,

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5 g of yeast nitrogen base, 20 g of bacto casitone, 1 g of sorbitan monooleate, 2 g of ammonium citrate, 5 g of sodium acetate, 2 g of  $K_2HPO_4$ , 0.05 g of  $MnSO_4$ , 0.1 g of  $MgSO_4$ . The cells were grown in the culture medium with continuous shaking on a rotary shaker (120 rpm) at 30°C.

The cultures of *L. delbrueckii* were grown in 500 ml conical flasks containing 200 ml of culture medium at 30°C. Prior to use for the experiments, the cells were harvested by centrifugation at 1000 g for 15 min. The  $\beta$ -glucosides were prepared as follows. A total of 2 mmol of substrate (0.2 mmol/flask) was added to ten 300-ml conical flasks containing 5 g of *L. delbrueckii* cells and 1 g of glucose in 100 ml of freshly prepared culture medium. The mixture was incubated with continuous shaking on a rotary shaker (120 rpm) for 5 days at 30°C. The reaction mixture was centrifuged at 8000 g for 15 min to remove the cells and the supernatant was extracted with *n*-butanol. The *n*-butanol fraction was purified by preparative HPLC on YMC-Pack R & D ODS column to give  $\beta$ -glucoside product.

To a solution containing 0.1 mmol of  $\beta$ -glucoside of capsaicin and 5 g of starch in 25 mM of sodium phosphate buffer (pH 7.0) was added 100 U of CGTase. The reaction mixture was stirred at 40°C for 24 h, and then the mixture was centrifuged at 3000 g for 10 min. The supernatant was subjected on to a Sephadex G-25 column equilibrated with water to remove CGTase. The fractions containing glycosides were purified by preparative HPLC on YMC-Pack R & D ODS column to give  $\beta$ -maltooligoside products.

### 2.3. Synthesis of Capsaicin $\beta$ -Melibioside and $\beta$ -Isomaltoside

A typical procedure to synthesize  $\beta$ -melibioside products is as follows. Hepta-*O*-acetyl- $\alpha$ -D-melibiosyl fluoride was prepared from octaacetylated maltoside and hydrogen fluoride by modification of Noyori's method. Under a nitrogen atmosphere,  $BF_3 \cdot OEt_2$  was added to a mixture of hepta-*O*-acetyl- $\alpha$ -D-melibiosyl fluoride (1.5 mmol), starting material (capsaicin, 1 mmol), and 1,1,3,3-tetramethylguanidine (3 mmol) in acetonitrile (20 mL) at room temperature. After the mixture had been stirred for 2 h, saturated aqueous sodium hydrogen carbonate was added. Organic materials were extracted twice with ethyl acetate, washed with saturated aqueous potassium hydrogen sulfonate and brine, dried, and concentrated. The residue was chromatographed on silica gel to give capsaicin hepta-*O*-acetyl- $\alpha$ -D-melibioside. Deacetylation of capsaicin hepta-*O*-acetyl- $\alpha$ -D-melibioside with 3% potassium carbonate in methanol afforded capsaicin  $\beta$ -melibioside. The other glycoside, *i.e.*,  $\beta$ -isomaltoside of capsaicin, was prepared by the similar procedure.

Spectral data of new compounds are as follows.

Capsaicin  $\beta$ -melibioside: FABMS:  $m/z$  652  $[M + Na]^+$ ;  $^1H$  NMR (400 MHz,  $CD_3OD$ ):  $\delta$  0.95 (6H, d,  $J = 6.8$  Hz, H-16, 17), 1.38 (2H, q,  $J = 7.6$  Hz, H-11), 1.62 (2H, q,  $J = 7.6$  Hz, H-10), 2.00 (2H, m, H-12), 2.20 (3H, m, H-9, 15), 3.22 - 3.82 (12H, m, H-2', 2'', 3', 3'', 4', 4'', 5', 5'', 6', 6''), 3.83 (3H, s,  $OCH_3$ ), 4.28 (2H, s, H-7), 4.41 (1H, d,  $J = 8.0$  Hz, H-1'), 4.75 (1H, d,  $J = 3.2$  Hz, H-1''), 5.30 - 5.41 (2H, m, H-13, 14), 6.80 (1H, dd,  $J = 8.0, 1.6$  Hz, H-6), 6.92 (1H, d,  $J = 1.6$  Hz, H-2), 7.08 (1H, d,  $J = 8.0$  Hz, H-5);  $^{13}C$  NMR (100 MHz,  $CD_3OD$ ): 23.2 (C-16, C-17), 26.5 (C-10), 30.3 (C-11), 32.0 (C-15), 33.3 (C-12), 36.9 (C-9), 43.5 (C-7), 56.5 ( $OCH_3$ ), 62.7 (C-6''), 68.4 (C-6'), 70.4 (C-4''), 71.0 (C-4'), 71.5 (C-2''), 72.2 (C-3''), 75.8 (C-2'), 76.4 (C-3', C-5''), 77.8 (C-5'), 100.5 (C-1'), 105.8 (C-1''), 113.3 (C-2), 118.2 (C-5), 121.2 (C-6), 127.7 (C-13), 135.1 (C-1), 139.0 (C-14), 147.0 (C-4), 150.5 (C-3), 176.0 (C-8). Calcd. for  $C_{30}H_{47}NO_{13}$ : C, 55.25; H, 7.26; N, 2.15. Found: C, 55.12; H, 7.20; N, 2.10.

Capsaicin  $\beta$ -isomaltoside: FABMS:  $m/z$  652  $[M + Na]^+$ ;  $^1H$  NMR (400 MHz,  $CD_3OD$ ):  $\delta$  0.95 (6H, d,  $J = 6.8$  Hz, H-16, 17), 1.38 (2H, q,  $J = 7.6$  Hz, H-11), 1.60 (2H, q,  $J = 7.6$  Hz, H-10), 1.99 (2H, m, H-12), 2.22 (3H, m, H-9, 15), 3.27 - 3.88 (12H, m, H-2', 2'', 3', 3'', 4', 4'', 5', 5'', 6', 6''), 3.83 (3H, s,  $OCH_3$ ), 4.28 (2H, s, H-7), 4.49 (1H, d,  $J = 7.6$  Hz, H-1'), 4.82 (1H, d,  $J = 2.4$  Hz, H-1''), 5.29 - 5.41 (2H, m, H-13, 14), 6.80 (1H, d,  $J = 8.0, 1.6$  Hz, H-6), 6.92 (1H, d,  $J = 1.6$  Hz, H-2), 7.09 (1H, d,  $J = 8.0$  Hz, H-5);  $^{13}C$  NMR (100 MHz,  $CD_3OD$ ): 23.2 (C-16, C-17), 26.5 (C-10), 30.3 (C-11), 32.2 (C-15), 33.3 (C-12), 36.9 (C-9), 43.5 (C-7), 56.5 ( $OCH_3$ ), 62.7 (C-6''), 68.5 (C-6'), 71.5 (C-4'), 72.0 (C-4''), 72.8 (C-5''), 73.4 (C-2''), 73.7 (C-3''), 75.2 (C-2'), 76.4 (C-3'), 78.0 (C-5'), 100.4 (C-1'), 106.0 (C-1'') 113.3 (C-2), 118.0 (C-5), 121.2 (C-6), 127.7 (C-13), 135.1 (C-1), 139.0 (C-14), 147.0 (C-4), 150.5 (C-3), 176.0 (C-8). Calcd. for  $C_{30}H_{47}NO_{13}$ : C, 55.25; H, 7.26; N, 2.15. Found: C, 55.20; H, 7.04; N, 2.09.

### 2.4. Suppressive Action on IgE Antibody Formation

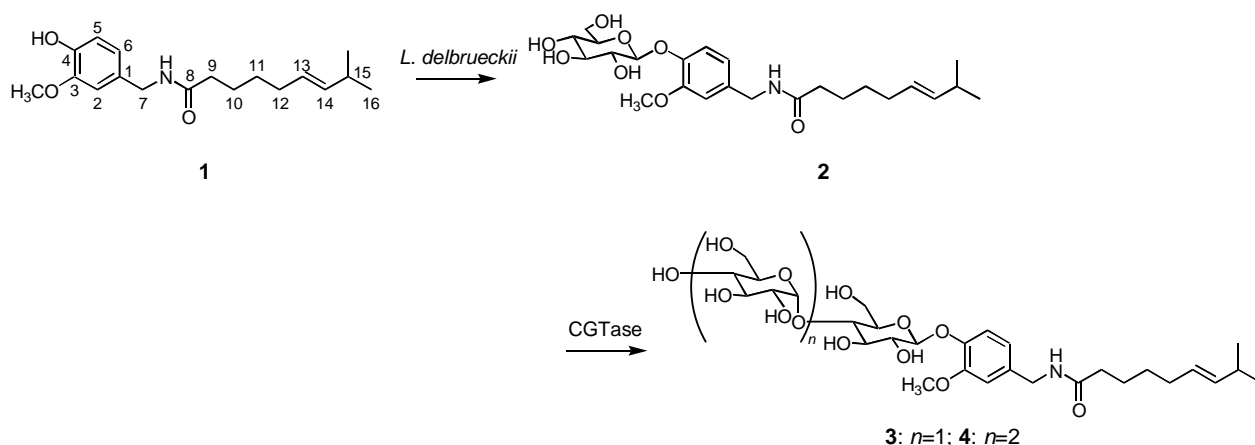
The inhibitory action of  $\beta$ -glycosides of capsaicin on IgE antibody formation was examined as follows. Glutenin was used as the antigen (1 mg/rat), and  $Al(OH)_3$  and pertussis vaccine were used as the adjuvants (20 mg and 0.6 ml/rat, respectively). Sensitization was made by injection of a mixture (0.6 ml) of the antigen and the adjuvant into the paws of each rat (male, *ca.* 200 g). Paw edema was measured 24 h after injection and the treated rats were divided in groups with an equal average swelling volume. Each sample was dissolved in physiological saline containing 10% Nikkol and the solution was injected daily into the rat for 11 d starting on the day of grouping. Hydrocortisone was used as the positive control. The amount of IgE was measured by the passive cuta-

neous anaphylaxis method on the 15th day [20]. The results were expressed as average of plasma IgE level of 7 rats administered a total of 10 mg/kg of each test compound.

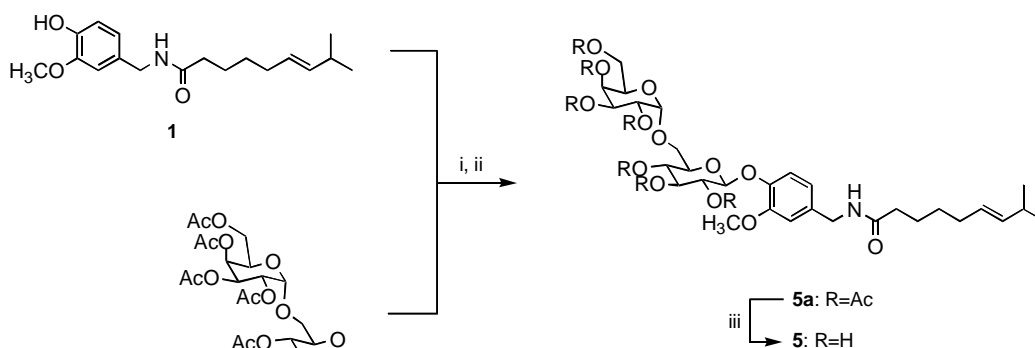
### 3. Results and Discussions

#### 3.1. Biocatalytic Synthesis of Capsaicin $\beta$ -Maltoside and $\beta$ -Maltotrioside

The biotransformation of capsaicin (**1**) was investigated using the cultured cells of *L. delbrueckii*. Cells of *L. delbrueckii* were incubated with capsaicin (**1**) in the presence of glucose for 5 days. Product **2** was isolated from *n*-butanol extracts of cell cultures by preparative HPLC (7% yield). The chemical structure of **2** was determined by spectroscopic methods such as HRFABMS,  $^1\text{H}$  and  $^{13}\text{C}$  NMR as capsaicin  $\beta$ -D-glucoside (**2**). Next, the glucoside product **2** was further glycosylated by cyclodextrin glucanotransferase (CGTase) to give two compounds **3** and **4** in 41 and 35% yield. The structures of products **3** and **4** were identified as capsaicin  $\beta$ -maltoside and capsaicin  $\beta$ -maltotrioside by HRFABMS,  $^1\text{H}$  and  $^{13}\text{C}$  NMR, H-H COSY, C-H COSY, and HMBC spectra. The synthetic route of capsaicin maltooligosides is shown in **Figure 1**.



**Figure 1.** Synthesis of capsaicin maltooligosides by *L. delbrueckii* and CGTase.



**Figure 2.** Synthesis of capsaicin  $\beta$ -melibioside. Reagents and conditions: (i)  $\text{BF}_3 \cdot \text{OEt}_2$ , 1,1,3,3-tetramethylguanidine; (ii) aq. sodium hydrogen carbonate; (iii) 3% potassium carbonate/methanol.

**Table 1. Suppressive action of capsaicin  $\beta$ -glycosides 2 - 6 on IgE antibody formation.**

Compound	IgE level <sup>a</sup>
None	382.2
2	152.5
3	177.6
4	395.0
5	155.1
6	170.4
Hydrocortisone	341.0

<sup>a</sup>The results were expressed as average of plasma IgE level of 7 rats administered a total of 10 mg/kg of each test compound.

The FABMS spectrum of **5** showed a peak at  $m/z$  652  $[M+Na]^+$  suggesting a molecular formula of  $C_{30}H_{47}NO_{13}$ . The  $^1H$  and  $^{13}C$  NMR data of the sugar moiety of **5** agreed with those of  $\beta$ -melibiose. Each signal in the NMR spectra of **5** was assigned by H-H COSY, C-H COSY, and HMBC analyses. The HMBC spectrum of **5** showed correlations between the proton signal at  $\delta$  4.75 (H-1') and the carbon signal at  $\delta$  147.0 (C-4), and between the proton signal at  $\delta$  4.41 (H-1'') and the carbon signal at  $\delta$  68.4 (C-6'), which confirmed that the inner  $\beta$ -D-glucopyranosyl residue was attached to the 4-hydroxyl group of capsaicin and that the second  $\beta$ -D-galactopyranosyl residue and the inner  $\beta$ -D-glucopyranosyl residue were 1,6-linked. Therefore, the structure of **5** was determined to be capsaicin  $\beta$ -melibioside. Compound **5** has not been identified previously.

Product **6** was assigned a  $Mr$  of 652  $[M + Na]^+$  in the FABMS spectrum, which suggested a molecular formula of  $C_{30}H_{47}NO_{13}$ . In the  $^1H$  and  $^{13}C$  NMR spectra of **6**, the coupling pattern of the sugar proton signals and the chemical shifts of the sugar carbon signals indicated that the sugar component in **6** was  $\beta$ -isomaltose. Correlations were observed in the HMBC spectrum between the proton signal at  $\delta$  4.82 (H-1') and the carbon signal at  $\delta$  147.0 (C-4), and between the proton signal at  $\delta$  4.49 (H-1'') and the carbon signal at  $\delta$  68.5 (C-6'). These results confirmed that the inner  $\beta$ -D-glucopyranosyl residue was attached to the phenolic hydroxyl group of capsaicin and that the second  $\beta$ -D-glucopyranosyl residue and the inner  $\beta$ -D-glucopyranosyl residue were 1,6-linked. Thus, compound **6** was identified as capsaicin  $\beta$ -isomaltoside, which is a new compound.

### 3.3. Anti-Allergic Activity of Capsaicin $\beta$ -Glycosides

The effects of capsaicin  $\beta$ -glycosides **2-6** on IgE antibody formation were investigated by *in vivo* bioassay using glutenin as an antigen [20]. The average of rat plasma

IgE level after treatment of glutenin with or without test compounds was examined. As shown in **Table 1**, capsaicin  $\beta$ -D-glucoside (**2**) and capsaicin  $\beta$ -maltoside (**3**) showed strong inhibitory action on IgE antibody generation. On the other hand, capsaicin  $\beta$ -maltotrioside (**4**) did not exhibit the inhibitory action on IgE antibody formation. Capsaicin  $\beta$ -melibioside (**5**) and capsaicin  $\beta$ -isomaltoside (**6**) also showed inhibitory action on IgE antibody formation.

## 4. Conclusions

The results of this experiment demonstrate that cultured cells of *L. delbrueckii* converted capsaicin into its glucoside and that CGTase glycosylate capsaicin glucoside to capsaicin  $\beta$ -maltoside and capsaicin  $\beta$ -maltotrioside. Also, capsaicin  $\beta$ -melibioside **5** and capsaicin  $\beta$ -isomaltoside **6** were successfully prepared by chemical syntheses in the presence of 1,1,3,3-tetramethylguanidine. Capsaicin  $\beta$ -glucoside, capsaicin  $\beta$ -maltoside, capsaicin  $\beta$ -melibioside, and capsaicin  $\beta$ -isomaltoside showed strong inhibitory action on IgE antibody generation. On the other hand, capsaicin  $\beta$ -maltotrioside did not exhibit antiallergic activity. It should be emphasized that capsaicin mono- and disaccharides would be useful functional food-ingredients which show anti-allergic activity toward glutenin. Further studies on the physiological activities of capsaicin glycosides are now in progress.

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